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The Fungal Association in a Colony of *Gastrodia sesamoides* R.Br.

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Abstract

AT Silverdale *Gastrodia sesamoides* behaves as an epiparasite on the roots of *Acacia melanoxylon*. A fungus occurs both as a root-inhabiting parasite on the *Acacia* and also as an endophyte in roots and rhizomes of the *Gastrodia*. In the orchid a digestion process takes place. The fungus is thought to be *Fomes mastoporus*.

OCCURRENCE OF *Gastrodia sesamoides*

Gastrodia sesamoides is growing in an area of a few square yards in one part of a 4-acre stand of *Acacia melanoxylon* R.Br. at Bankside, Silverdale. The original trees were imported from Australia 80 or more years ago and planted out in what was at the time manuka scrub country. Natural regeneration of the *Acacia* has taken place resulting in a well-established stand. Where some of the oldest trees have been milled, the stumps now carry the large sporophores of a bracket fungus, *Fomes mastoporus* (Lev.) Cooke, and a circle of adjacent trees have died. In some cases a tree-trunk has died only on the side facing the stump, thus suggesting that the causative agent is a root-infecting fungus spreading outwards from its base in the old stump through contact between roots below ground. The soil in the area is very shallow, consisting of a brown, organic horizon 8 to 10cm deep overlying a stiff, grey clay. Fine rootlets of the *Acacia* trees penetrate throughout the organic layer, and lying horizontally amongst them are found tubers of the *Gastrodia*. In November the tall, flowering stems of the orchid grow up above the soil. Illustrations of these and of the structure of the flowers have been given by Hatch (Hatch 1949, 1954).

Gastrodia sesamoides has been reported from other parts of New Zealand (Cheeseman 1925, Hatch 1949) and also from Australia (McLennan 1959). In many areas in New Zealand where it formerly occurred the original vegetation is now known to have been destroyed and no record has been kept of the plants with which the *Gastrodia* was associated. However, V. M. Scott in correspondence has mentioned that at Takaka in 1952 and 1953 she found *Gastrodia sesamoides* in mixed native forest and also amongst *Pteridium* on a hillside where the former forest cover had been burnt some 30 years previously. On visiting the forest area

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again this year she noted enormous bracket fungi on two ancient prostrate logs of *Laurelia novae-zelandiae* A. Cunn. In 1959 A. J. Healy found a colony at Foxton Beach growing amongst *Coprosma acerosa* A. Cunn., *Leptocarpus simplex* A. Rich., and *Scirpus nodosus* Rottb. in a damp hollow between the dunes.

Collection and Treatment of Material

Tubers were collected from Bankside on November 6, 1962, as the new season's shoots were emerging from the soil, and on February 22, 1963, as the new tubers for the next flowering season were enlarging. Unfortunately the area contains only about a dozen flowering plants and is subject to interference by birds and stock. An attempt was made to select what might be regarded as typical plants, but it was not possible to check with others without destroying the whole colony. The tubers, together with adjacent roots of *Acacia melanoxylon,* were killed and fixed immediately in formalin-acetic-alcohol. Selected portions were embedded in paraffin and sectioned at thicknesses of 8 μ to 20 μ. Double staining with safranin and fast green proved satisfactory for showing detail in the sections, although combinations of chrysoidin and fast green and of crystal violet and erythrosin were tried also. Lignification was determined by the staining reaction of phloroglucin followed by strong hydrochloric acid, and suberization by staining with Sudan III. In order to trace the distribution of hyphae in *Acacia* roots and stems hand-cut sections were stained in cotton-blue and mounted in lacto-glycerine.

The identification of the orchid species was determined from flowers collected at Bankside in December. These were dissected and compared with pressed specimens from the herbarium of Botany Division, D.S.I.R.

The Underground System of *Gastrodia sesamoides*

The tubers for the most part are those of one season only, as older ones tend to rot away in the autumn. They occur singly (Fig. 1) or occasionally as 2 to 4 joined together in a sympodial rhizome system extending horizontally in the soil (Fig. 2). Each tuber is up to 8cm long, 3cm broad and 2cm deep. At intervals along their length they carry the torn remnants of scale leaves and lateral buds, some of which will form new tubers. In the autumn there may occur on the tubers one or more slender roots 1mm in breadth and up to 13cm in length, but the tips of these die back, and in the summer any roots present are shorter, often coralloid or bearing very short, coralloid laterals. The roots are distinguishable from young lateral rhizomes by the lack of scale leaves and the presence of a root cap. Regions of fungal infection are evident on the surface of both roots and rhizomes as brownish or black, irregular, pock-like marks 2–10mm in diameter. Interwoven with the *Gastrodia* plants grow slender roots of the *Acacia* trees, some of the finer rootlets of diameter 0.2–0.3mm lying closely appressed to the surface of either root or tuber or occupying a position between the lobes of the coralloid roots.

Anatomy of the Rhizome of *Gastrodia sesamoides*

In transverse section the mature tuber is either circular or else flattened in the plane of the ground to a somewhat oval shape. Externally there is a protective region, 1 to 3 cells in depth, composed of flattened cells with walls brownish due to the presence of tannin-like compounds (Fig. 5). Beneath this is a subepidermal layer of isodiametric cells showing suberization of the radial and outer tangential walls and in some cases of the inner tangential walls also. The outer cortex 3 to 4 cells in depth is a thin-walled tissue of small cells most of which

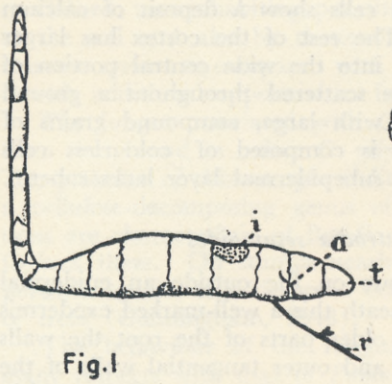

Fig.1

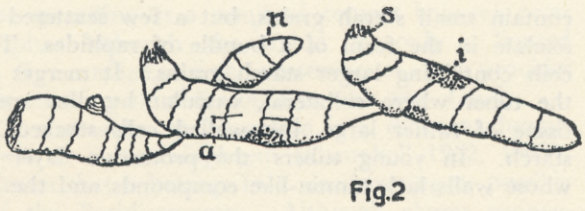

Fig.2

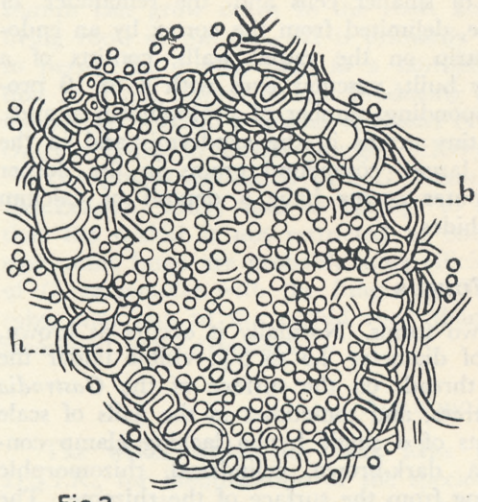

Fig.3

Fig.4

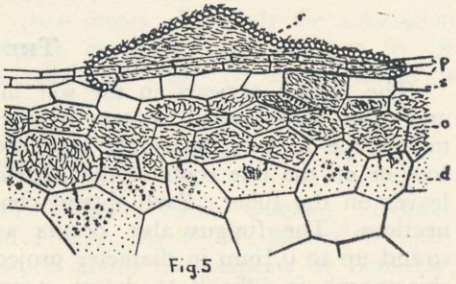

Fig.5

FIG. 1.—Plant of *G. sesamoides* in November. × ½. a—*Acacia* root; i—area of fungal infection; r—*Gastrodia* root; t—tuber.

FIG. 2.—Tuber system of *G. sesamoides* in February. × ½. a—*Acacia* root; i—infected area; n—new tuber; s—scar of old flowering stem.

FIG. 3.—Transverse section of a rhizomorph. × 800. b—brown-walled hyphae; h—hyaline hyphae.

FIG. 4.—Transverse section of the outer part of a *Gastrodia* root showing infection of the exodermis. × 400. e—epidermis; x—exodermis.

FIG. 5.—Transverse section of a *Gastradia* tuber showing infection from a rhizomorph. × 160. d—digestive layer; o—outer cortex; p—protective layer; s—subepidermal layer; r—rhizomorph.

contain small starch grains, but a few scattered cells show a deposit of calcium oxalate in the form of a bundle of raphides. The rest of the cortex has larger cells containing larger starch grains. It merges into the wide central portion of the tuber where collateral, vascular bundles are scattered throughout a ground tissue of rather large, thin-walled cells stocked with large, compound grains of starch. In young tubers the protective layer is composed of colourless cells whose walls lack tannin-like compounds and the subepidermal layer lacks suberin.

Anatomy of the Root of *Gastrodia sesamoides*

A transverse section of the orchid root shows on the outside an epidermal layer of flattened cells lacking root hairs, and beneath this a well-marked exodermis of larger, more isodiametric cells (Fig. 6). In older parts of the root the walls of the epidermal cells are brown and the radial and outer tangential walls of the exodermal cells are suberized. The rest of the cortex is 8 to 12 cells in width, the outermost 2 or 3 layers consisting of smaller cells and the remainder of larger, thin-walled cells. The central stele, delimited from the cortex by an endodermis with a well-marked casparian strip on the radial walls, consists of a pericycle 2 cells in width and a radially built, vascular core with 4 to 10 protoxylem strands alternating with a corresponding number of protophloem strands. Starch is present in the root, stored as tiny grains in the exodermis and in the small cells of the outer cortex and as larger, compound grains in the deeper cortical cells. Occasional cells of the outer cortex have a deposit of calcium oxalate in the form of a bundle of raphides.

The Fungus

The fungus appears in the soil in two forms. Normally it occurs as brown, and often thick-walled, septate hyphae of diameter 2.5 to 5 μ, visible under the microscope as long, sparingly branched threads on the surface of the *Gastrodia* root or on the fine roots of the *Acacia* trees, and sometimes in the axils of scale leaves on the tuber. Each thread consists of a single hypha lacking clamp connections. The fungus also occurs as a dark-brown, cylindrical, rhizomorphic strand up to 0.1mm in diameter projecting from the surface of the rhizome. The rhizomorph is difficult to detect owing to its fragile nature and its resemblance to the fine *Acacia* rootlets amongst which it lies. In section it shows a central core of hyaline hyphae running lengthwise, enclosed by brown-walled hyphae which are arranged in a characteristic pattern (Fig. 3). Fringing, brown hyphae lie more or less parallel in a lengthwise direction or radiate outwards, but the innermost ones by branching at a wide angle and interweaving construct an openly braided meshwork. The hyphae of the rhizomorph are 2.5 to 3.5 μ in diameter.

A similar fungus occurs as a white weft under the bark of some of the *Acacia* trees and may build up into white, mycelial strands, 0.05mm in diameter, in the region of the cambium. These strands have the outermost hyphae arranged in the form of a meshwork as in rhizomorphs in the soil, and can be traced along the trunk to the sporophores of *Fomes mastoporus*. In affected trees the bark lifts easily from the xylem beneath.

The Fungal Infection of the Orchid

Infection of the Root

Roots collected in November and in February showed active invasion by the fungus, and all phases of the process were in evidence. Single hyphae of the fungus penetrate along the radial walls in young parts of the root and enter a

few of the epidermal cells. Once inside the cell they ramify within the cell cavity as thin-walled hyphae, of diameter 1.5 to 2.5μ, lacking clamp connections. The nucleus of the infected cell at first enlarges and then together with the cytoplasm disappears. The hyphae may persist either in the thin-walled condition or in a wider form up to 3.5μ in diameter with thicker walls of a brown colour. Some hyphae penetrate into single cells of the exodermis growing towards the nucleus where their tips may break down, resulting in the release of fungal cytoplasm into the invaded cell. Invasion of the exodermis is accompanied by alteration of the penetrated tangential wall which now appears much thicker and yellowish-brown in colour (Fig. 4). Often cellulosic sheaths impregnated with material similar to that in the altered wall are deposited around the hyphae at the point of entry and remain projecting into the exodermal cell as papillose outgrowths (röhrentüpfel) staining deeply with safranin as does the penetrated wall itself.

The infection may be halted at this stage and the hyphae in the exodermal cell disappear. At other times the living contents of the exodermal cell are destroyed while adjacent cells of the outer cortex react in the same way as does a typical exodermal cell. However, the usual course of events when hyphae penetrate to deeper tissue is that an exodermal cell which may be called a passage cell becomes occupied by fine, loosely coiled hyphae, some of which penetrate into the underlying tissue (Fig. 6). Surrounding exodermal cells remain free of the fungus except that on rare occasions there are two adjoining passage cells.

Once through the exodermis the hyphae spread laterally in the two or three layers of small cells of the outer cortex and gradually extend to a varying distance ranging from 3 to commonly as much as 10 to 15 or more cells from the point of entry (Fig. 6). Neighbouring infection areas may merge, with the result that the fungus may be present in most or all of the outer cortical cells in any transverse section. In each infected cell there develops a coil of hyphae which at first are thin-walled and of diameter 1.5 to 2μ. When there is an extensive infection, hyphae occupying the centre of the coil become thick-walled and up to 4 μ in diameter. They connect from one cell to the next providing the structural framework of the system, and persist after the fine hyphae have collapsed. Starch disappears from the cells and, wherever calcium oxalate occurs, it also disappears, the idioblastic cells remaining recognisable by the few hyphae present within them. The nucleus of each invaded cell enlarges and the nucleolus becomes prominent, then the nucleus becomes irregular in outline, loses its affinity for stains, and together with the cytoplasm disappears. The walls of the invaded cortical cells become impregnated with brownish, tannin-like compounds and those in the outer part become lightly lignified as well. Very occasionally lignified sheaths are present on hyphae at the point of entry into a cell.

Cells of the root situated on the inner side of the tangentially spreading infection constitute a digestive layer (Fig. 6). Already larger than cells of the outer cortex they increase in size radially until they are as much as four times their original diameter and also show prominent pitting of the enlarged walls. Hyphae growing radially inwards from the outer cortex penetrate the wall and branch into many fine threads which take a convoluted path as they grow towards the nucleus which meantime has become enlarged and very granular with a conspicuous nucleolus. Starch gradually disappears from the cell. The tips of the hyphae swell enormously as they accumulate granular cytoplasm, then alter in staining properties and appear as spherical bodies, retentive of safranin stain, lying in the cytoplasm of the digestive cell either singly or in masses enclosed by a membrane. The nucleus becomes lobed or amoeboid and the nucleolus may fragment, but the extreme deformation which occurs in digestive cells of the rhizome of *Gastrodia cunninghamii* and *G. minor* (Campbell 1962, 1963) was

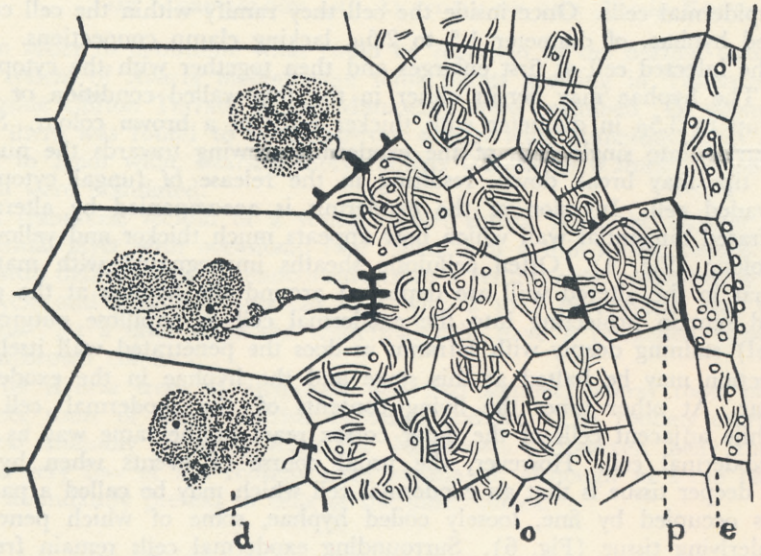

Fig.6

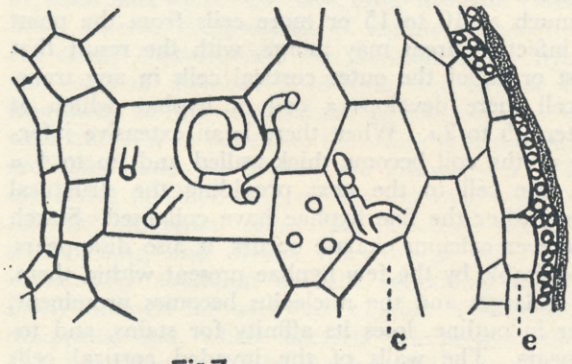

Fig.7

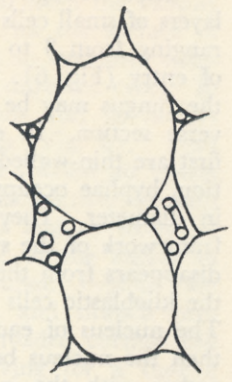

Fig.8

FIG. 6.—Transverse section of the outer part of a *Gastrodia* root showing fungal infection. × 380. e—epidermis; d—digestive layer; o—outer cortex; p—passage cell in exodermis.

FIG. 7.—Transverse section of the outer part of an *Acacia* rootlet showing fungal infection. × 400. e—epidermis; c—cortex.

FIG. 8.—Transverse section of a part of the cortex of a fine *Acacia* root showing intercellular hyphae. × 400.

not observed. Later the nucleus gradually assumes a more spherical shape though still enlarged. Slowly the spherical bodies are absorbed until they disappear completely. Remnants of the coiled hyphal threads persist for some time, surrounded where they entered the cell by a collar formed of cell-wall material. The penetrated wall itself becomes altered in nature and is now impregnated with tannin-like compounds but all other walls of the digestive cell remain of cellulose as formerly.

Eventually in old infection areas the walls of invaded cells in the outer cortex appear dark brown, and the empty hyphal threads of the endophyte have disappeared. The affected region tends to collapse or scale off, and deeper cells may also die off and develop brown walls. So extensive may be the necrotic lesion that the distal portion of the root shrivels.

Infection of the Rhizome

Tubers collected in November showed no active infection by the fungus, although in roots collected at the same time the endophyte was present. However, the site of previous infections was indicated on the tubers by rough areas left where the surface layers had scaled off or by cavities beneath the external protective region left in the position where invaded cells had collapsed. On the other hand in rhizomes collected in February active infection was in evidence, sited frequently in the axils of the scale leaves.

In young tubers infection occurs from single hyphae, the fungus penetrating only to the subepidermal layer or penetrating to the outer cortex where it extends laterally to a varying extent. The invaded cells react in the same way as in the root. Their walls eventually turn brown but only a few of the outermost cortical ones become lignified. Deeper cells may also develop brown walls and die off. Eventually the region affected by the fungus collapses leaving a cavity beneath the protective layer.

In older tubers infection was found to have occurred also from rhizomorphs, a condition which was not observed in roots. In this latter type the rhizomorph attaches itself to the tuber by a more or less hemispherical disc (Fig. 5). Brown-walled hyphae of meshwork arrangement make the outer covering of the disc and also occupy a circle of adjacent cells of the protective layer so giving a comparatively firm attachment. Hyaline hyphae from the central portion of the disc fill the cells of the protective layer beneath them with coiling hyphae. The hyaline hyphae also penetrate into a subepidermal cell where they form a dense coil completely filling the cell. Entry is accompanied by a thickening of the outer tangential wall of the invaded cell. Subepidermal cells in the immediate neighbourhood show no evidence of the fungus, but supplementary entry points may occur at a distance of 6 to 12 cells from the main infection, initiated by hyphae which have grown outwards from the rhizomorph along the surface of the tuber. From the passage cell in the subepidermis with its tight, fungal coil hyphae extend into the outer cortex, often ensheathed as they penetrate the wall, and then spread tangentially for a considerable distance in a band 2 to 3 cells wide. In each invaded cell a coil of hyphae develops and the protoplast disappears. Cells on the inner edge of the band function as digestive cells where the process of digestion corresponds with that found in the root, except that radial enlargement of the cells is less pronounced and the nucleus becomes deeply constricted into three or more portions as in corresponding cells in the rhizomes of *Gastrodia cunninghamii* and *G. minor*. The infection persists for a longer time and is much more extensive than that produced in the rhizome by single hyphae.

THE MORPHOLOGY AND ANATOMY OF THE *Acacia* ROOTS

In order to determine what could be regarded as the normal structure of the root system of *Acacia melanoxylon* some plants growing in Tokomaru silt loam alongside Clifton Terrace in the Manawatu were examined. In young plants 1m high there was found to be a tap-root system with numerous long, lateral roots 1mm to 2mm wide, bearing short rootlets 0.2mm to 0.3mm in width, some of which were nodulated.

A transverse section of a young rootlet shows, from the outside inwards, an epidermal layer with occasional root hairs, then a thin-walled cortex 5 to 7 cells in width, delimited by an endodermis from a central stele consisting of a uniseriate pericycle and a narrow vascular core with 2 to 5 protoxylem and protophloem strands. At a later stage the epidermal cells have a thickened outer wall of a yellow-brown colour.

The *Acacia* trees at Silverdale also had slender, lateral roots bearing short rootlets, some of which carried bacterial nodules. The rootlets corresponded in general anatomy to those from the Manawatu. However, near the *Gastrodia* plants very few of the rootlets were nodulated, although in some cases bacteria were abundantly present in cells of the inner cortex.

THE FUNGAL INFECTION OF THE *Acacia*

An examination of sections of fine roots and rootlets collected from the vicinity of *Gastrodia* tubers or roots, and in some cases from grooves on the surface of these, showed the presence of septate, fungal hyphae up to 5μ in diameter without clamp connections. The location of the hyphae differs in different cases. In fine roots of diameter 1 to 2mm the hyphae have brown walls and lie mainly lengthwise in the intercellular spaces between the cortical cells (Fig. 8). They were not seen to penetrate the walls. On the other hand, in the rootlets air spaces are absent and the hyphae are intracellular (Fig. 7). They accumulate beneath the layers of thickening on the external walls of the epidermis and at times inside the epidermal cells, then penetrate deeper, often starting in the region of a root hair, and invade the cortical cells. Thin-walled, hyaline hyphae are particularly abundant in cells containing endophytic bacteria and branch profusely amongst the bacterial colonies. The protoplast of each invaded cortical cell disappears and, where bacteria are present, these die off leaving for a time a granular deposit. Later the infected part of the cortex may collapse leaving a cavity beneath the surface layer. Hyphae do not readily penetrate the endodermis but in the two cases where they were seen in the pericycle a phellogen had arisen beneath the damaged cells so impeding further penetration by the fungus.

DISCUSSION

Although *Gastrodia sesamoides* has been reported from several localities in New Zealand, growing amongst native vegetation as well as occurring at Silverdale in a stand of *Acacia melanoxylon,* it nowhere seems to be abundant. It differs from the two other indigenous species of the genus not only in the structure of the flower but also in possessing roots on its fleshy rhizomes, as well as in several minor respects.

Like the related species, *G. sesamoides* is interpreted as being an epiparasite on the roots of other plants. By a digestion process in both its roots and its rhizomes the orchid obtains nutriment from an endophytic fungus which, in the locality investigated, occurs also in *Acacia melanoxylon,* where it behaves as a

specialised, root-inhabiting parasite spreading as a result of contact between roots in the soil and also by rhizomorphs. Although the orchid has only a limited rhizome system as compared with *G. cunninghamii* the possession of roots provides it with additional opportunities for making suitable contacts with the *Acacia.*

The fungus associated with *G. sesamoides* at Silverdale is a different fungus from that found in either *G. minor* or *G. cunninghamii* (Campbell 1962, 1963). The presence of rhizomorphs indicates that it belongs to a wood-rotting or possibly a cellulose-decomposing genus of the Basidiomycetes. Although clamp connections are characteristic of Basidiomycetes, a few genera such as *Fomes* are known to lack these. On stumps nearby there grow the sporophores of *Fomes mastoporus,* a species of widespread occurrence in New Zealand producing a white rot of trees (Cunningham, 1948).

The behaviour of the fungus associated with the *Gastrodia* corresponds in many respects with that of *Fomes annosus* (Rishbeth, 1950, 1951 a, b). In this latter species a mycelium originating by germination of spores on freshly-cut stumps can grow down to the roots and then spread through contact between living roots in the soil. It is primarily a root-inhabiting parasite, although able to persist for a long time as a saprophyte in the stump. Rishbeth has shown that it is not always disease producing and that it can infect *Pteridium.* It is already known that a species of *Fomes* is associated with another non-green orchid, *Galeola hydra,* in Luzon (Burgeff, 1959).

The fungus associated with *G. sesamoides* in Australia has clamp connections (McLennan, 1959) and so belongs to a different genus. This would indicate that the orchid is not restricted to one particular fungus. Should this be the case, some fungi are probably more efficient than others. Certainly the association of *G. sesamoides* with the fungus at Silverdale results in more damage to the tuber and also to the orchid root than is the case when *G. cunninghamii* is associated with *Armillaria.*

ACKNOWLEDGMENTS

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